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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)

1- File Copy

*U S GPO 2000-473-000/44602

Application No.	Applicant(s)
09/359,672	BLACKBURN ET AL
Examiner	**Art Unit**
Quang Nguyen	1632

Office Action Summary

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED. (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 July 2001*

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27,29-40 and 42-50* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27,29-40 and 42-50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a)☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)
2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3)☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4)☐ Interview Summary (PTO-413) Paper No(s). _____
5)☐ Notice of Informal Patent Application (PTO-152)
6)☐ Other: _____

DETAILED ACTION

Continued Prosecution Application

The request filed on July 09, 2001 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/359672 is acceptable and a CPA has been established. An action on the CPA follows.

Claims 1-27, 29-40 and 42-50 are pending in the present application.

Claim Objections

Claim 27 is objected to because of the following informalities: the phase "to any claim 17" is not proper. There is only one claim 17. Appropriate correction is required.

Claim 31 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. This is because claim 29 already contains all the embodiments of claim 31. Please note that ES, EC or EG cell is an embryonic cell.

Sequence compliance

The disclosure is objected to because of the following informalities: The specification contains sequence listings. The nucleotide sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 (See page 31 of the instant application). Any amino

acid sequence having more than 3 amino acid residues or any nucleotide sequence having more than 9 nucleotides, a SEQ ID NO. must be assigned to each. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

Appropriate correction is required.

Written Description

Claims 1-16 and 29-50 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed. "Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

Applicant's invention is drawn to an *in vitro* method of expressing a DNA in a pluripotent cell, an assay for the effect of presence in a pluripotent cell of a protein or

polypeptide or other product of DNA expression. a method of investigating the properties of a DNA sequence comprising expressing in a pluripotent cell a composite DNA, and an ES, EC or EG cell comprising the vectors of the present invention. Apart from the disclosure of the mouse embryonic stem (ES) MG1.19 cells, the instant specification fails to disclose the availability of ES or embryonic carcinoma (EC) derived from other species as encompassed by the instant claims for carrying out the present invention. Additionally, the state of the art was such that at the effective filing date of the present application, the accessibility and availability of ES or EC cells derived from species other than the mouse is not readily available (Mullins et al.: J. Clin. Invest. 98:S37-S40, 1996; Moreadith et al., J. Mol. Med. 75:208-216, 1997). The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants' filing date. Furthermore, the instant specification fails to teach a sufficient number of ES or EC cells comprising the vectors of the present invention to represent the full scope of the present invention. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc., 48 USPQ2d 1641, 1646 (1998). The skilled artisan cannot envision the detailed structure of non-mouse ES or non-mouse EC cells and methods of using the same. Therefore conception is not achieved until reduction to practice has occurred. regardless of the

complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-27 and 29-50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for ***in vitro*** methods of expressing a DNA in a mouse ES cell, mouse EC cell or an EG cell, of assaying for the effect of presence in said cells of a protein or polypeptide or other product of DNA expression and for screening a library of cDNAs in said cells; a vector for transfection of said cells and an isolated mouse ES, mouse EC or an EG cell transfected with the same with the following limitations, the viral replication factor is limited to polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors or SV40 large T antigen and the episomal vector contains an appropriate viral origin of DNA replication that is

recognized and activated by a viral replication factor, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The instant claims encompass methods of using any and all pluripotent cells, including ES and EC derived from any and all species, as well as any and all ES or EC cells transfected with the vectors of the present invention. The instant specification is not enabled for such a broadly claimed invention for the reasons already set forth in the Written Description section above. Basically, apart from the specific disclosure of utilizing mouse embryonic stem cells in the present claimed invention, the specification fails to provide guidance for a skilled artisan on how to obtain and manipulate ES and EC cells derived from sources other than the mouse. It has been noted that the transgenesis art utilizing the ES cell technology is limited to the mouse system because only "putative" ES cells exist for other species (Moreadith et al. J Mol Med 75:208-216, 1997, page 214, Summary). In addition, Seamark (Reproduction, Fertility and Development 6:653-657, 1994) reported that totipotency for ES cell technology in many

livestock species has not been demonstrated (page 6, abstract). Additionally, Mullins et al. (J. Clin. Invest. 98:S37-S40, 1996) reported that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated" (page S38, column 1, first paragraph). Thus, with the lack of guidance provided by the specification, it would have required undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

With respect to the method claims relating to the utilization of the episomal vector system of the present application, as written the claims encompass the scope that any and all second vectors are able to be replicated extrachromosomally by any and all replication factors. The instant specification is not enabled for such a broadly claimed invention because there is no evidence of record in the present application or in the prior art indicating that any replication factor is able to transactivate any second vector without the presence of an appropriate viral origin of replication that is recognized and activated by the replication factor. Since the presence of an appropriate viral origin of replication that is recognized by a viral replication factor is essential for the maintenance and extrachromosomal replication of the second vector, it has to be recited in the claims. Additionally, it is unclear what other replication factors apart from the viral replication factors disclosed by the instant specification and those known in the art, are involved in the maintenance and extrachromosomal replication of a vector in a transfected cell. Since the instant specification fails to provide sufficient guidance

regarding to this matter, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed.

Regarding to the breadth of claims encompassing any and all functional variants, analogues and derivatives of the viral replication factor selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors and SV40 large T antigen. the specification does not provide sufficient guidance on which variations or modifications to be made on the aforementioned viral replication factors, such that these variants, analogues and derivatives are still functionally active. Moreover, the claims contain an embodiment associated with a high degree of unpredictability to make and uses. As is well recognized in the art, any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. Similarly, the present disclosure offers no guidance as to which regions of the viral replication factor molecule would be tolerant of alteration and which would not, which "particular" encoded amino acid changes (substitution, deletion or insertion) at which position and in which combinations, such that the variant encoded viral replication factors are still functional. In discussing peptide hormones, Rudinger has stated that "The significance of particular amino acids and sequences for different aspects of biological activity can not be predicted a priori but must be determined from case to case by painstaking experimental study (Page 6, first sentence of Conclusions *In* J.A. Parsons, ed. "Peptide hormones", University Park Press, 1976). This unpredictability is further underscored by the fact that the relationship between the sequence of a peptide and its tertiary structure (or its activity) is not well understood and

is not predictable (Ngo et al., *In* K. Merz et al., ed. "The protein folding problem and tertiary structure prediction", Birkhauser, 1994, 491-495). Therefore, without sufficient guidance provided by the instant specification, it would again require undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

With respect to claims in which there is no recitation of cultured cells or isolated cells or *in vitro*, Examiner interpretes the claims broadly to encompass both ***in vitro*** and ***in vivo*** situations. The instant specification is not enabled for such a broadly claimed invention, because the specification fails to provide any guidance or direction for a skilled artisan on the make and use of any claimed methods or genetically modified pluripotent cells ***in vivo***. The specification provides teachings exclusively on the construction of vectors and their applications in tissue cell cultures. The nature of claims encompassing the *in vivo* scope would fall within the realm of gene therapy which at the effective filing date of the present application was immature and highly unpredictable. The instant specification fails to provide guidance for a skilled artisan on how to overcome obstacles known in the gene therapy art. Issues such as vector targeting, adverse host immune responses directed against administered recombinant vectors, the fate and *in vivo* expression level of the transgene provided by the delivered vectors, routes of delivery, and others have not been addressed by the instant specification. Given the lack of such guidance, it would have required undue experimentation for one skilled in the art to make and use the broadly claimed invention.

Accordingly, due to the lack of sufficient guidance provided by the instant specification regarding to the issues set forth above, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 5, 6, 12-16, 24-27, 35 and 37-50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The article "A" in claims 2-16, 18-27, 30-32, 34-35, 38-40 and 42-50 renders the dependent claims indefinite because the scope of these claims can not be clearly determined. This is because it is unclear which method of claim 1, which vector of claim 17, which ES, EC or EG cell of claim 29, which assay method of claim 33 or which method of claim 37 that these dependent claims are referred to. To obviate this rejection, the article - - The - - should be used instead.

In claim 3, the phrase "functional variants, analogues and derivatives thereof appropriate to the cell species" renders the claim indefinite. This is because it is unclear under which conditions these functional variants, analogues and derivatives are appropriate or not appropriate to the cell species. The metes and bounds of the claim can not be clearly determined. Clarification is requested.

In claims 5 and 27, the phrase "the selectable marker is an antibiotic resistance gene" is unclear. This is because the selectable marker is a polypeptide or protein or a gene product, and not a gene. Clarification is requested.

In claim 6, it is unclear what is encompassed by the phrase "is adapted to receive a DNA". How is the vector adapted to receive a DNA? Apart from inserting a DNA into a restriction site downstream of a promoter that is operably linked to the inserted DNA. how is the vector adapted to receive a DNA? The metes and bounds of the claim can not be clearly determined. Clarification is requested.

In claims 12, 13, 15, 24 and 25, the phrase "the DNA codes for a polypeptide or protein" renders the claims indefinite. This is because it is unclear which DNA does it refer to? It is noted that in claims 1 and 27, the vector contains two DNA sequences, one that codes for a selectable marker, and the other does not code for a selectable marker. Which one? Clarification is requested.

In claims 14 and 26, the phrase "the promoter is inducible" renders the claims indefinite because it is unclear which promoter, one that operably linked to a DNA that codes for a selectable marker or one that operably linked to a DNA that does not code for a selectable marker, that the phrase refers to. Clarification is requested.

In claim 16, the phrase "replication of the second vector can be prevented by a site specific recombinase" is unclear. This is because by preventing the replication of the second vector, then how does this limitation relate to the expression of a DNA in a pluripotent cell as recited in the preamble of claim 1 from which claim 16 is dependent upon? Clarification is requested.

Claim 35 recites the limitation "two factors" in line 2 of the claim. There is insufficient antecedent basis for this limitation in the claim. The only factor that claim 33 from which claim 35 is dependent upon is a replication factor. Additionally, there is an improper Markush language in the phrase "each factor being independently selected from a protein, a polypeptide and another product of DNA expression". The substitution of the term - - or - - for "an" should obviate the improper Markush language.

In claim 37 and its dependent claims 39-50, it is unclear what is encompassed by the phrase "investigating the properties of a DNA sequence". To which extent is the investigation of the properties of a DNA sequence? It is also not apparent of the linkage between the recited steps (i)-(iii) with the preamble of claim 37. Furthermore, it should be noted that merely investigating the properties of a DNA sequence without any specific property of the DNA sequence needed to be investigated is just an invitation for further experimentation. The metes and bounds of the claims can not be clearly determined.

In claim 39, it is unclear what is encompassed by the term "disabling", and therefore it renders the claim indefinite. How is the DNA disabled? The metes and bounds of the claim can not be exactly determined.

Claim 48 recites the limitation "step (a)" in line 1 of the claim. There is insufficient antecedent basis for this limitation in the claim. In claim 37 from which claim 48 is dependent upon, there is no recited step (a). The metes and bounds of the claim can not be clearly determined. Clarification is requested.

In claims 49 and 50, there is a lack of step or steps linking the steps of claim 37 to the identification of a DNA coding for a cell surface or secreted protein, and to the identification of a cell surface or secreted protein as recited in the preambles of claims 49 and 50, respectively. How are DNAs coding for a cell surface or secreted protein or their expressed gene products identified? Clarification is requested.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-5, 8-12, 17-23, 27, 29-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7).

Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and it does not express the replication factor polyoma large T antigen due to a 1249-bp deletion in the coding region of the large T gene (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids,

please refer to Fig. 1. Because the PGKhphΔLT20 vector plasmid still contains a functional promoter operatively linked to a DNA sequence encoding the large T segment with a large deletion in its coding sequence (not a selectable marker), the mRNA message for this mutant large T antigen is still expressed in the transfected cells. There is no requirement for the encoded gene product of the second DNA to be produced in the transfected cells. Therefore, the teachings of Gassmann et al. meet all the recited elements in the claims, and thus the reference anticipates the instant claimed invention.

Claims 17-22, 24-25 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Carstens et al. (Gene 164:195-202, 1995).

The claims are directed to a vector for transfection of a pluripotent cell *in vitro* with specific limitations recited in the claims. For a composition claim, it should be noted that the intended use is not given any patentable weight in view of the prior art.

Carstens et al. disclosed a system that allows functional cloning of regulatory genes by the expression of libraries of cDNA inserts either in the sense or antisense direction using Epstein-Barr virus-based expression vectors. The disclosed system is designed to identify genes that are part of or act upon the anchorage signal transduction pathway. The system is comprised of two components (a) the library expression vectors, CMV-EL and C1E-EL, containing EB*ori*P for replication in EPNA-1 expressing cells; and (b) the EPNA-1-producing cell line BB-5, a derivative of the immortalized, non-tumorigenic and anchorage dependent human fibroblast cell line, MSU1.1. BB-5

cells supported the episomal replication of CMV-EL and C1E-EL and allowed the recovery of the vector from Hirt lysates of transfected BB-5 cells (See abstract). The vectors do not contain the EBNA-1 gene, but they comprise the sequence of hygromycin B (Hy^R) marker driven by the HSV-TK minimal promoter (See Fig. 1 and page 198, lines 4-6). Transcription of a cDNA insert in the multiple cloning site (MCS) is driven either by the cytomegalovirus (CMV) immediate early promoter/enhancer (CMV-EL) or by the CMV immediate early promoter/enhancer containing an additional bp of first exon sequences of the CMV immediate early gene (C1E-EL). Different cDNA inserts can be inserted into the vectors. Carstens et al. further stated that C1E-EL was specifically designed to allow a high level transcription of mRNA from cDNA libraries inserted in the antisense orientation (page 198, column 1, lines 16-18 of the first full paragraph). Both vector constructs have been tested by inserting a luciferase cDNA into the MCS (page 198, column 1, lines 18-20 of the first full paragraph). Additionally, Carstens et al. taught the clone BB-5 was derived from the transfection of human fibroblast MSU1.1 cells with an EBNA-1 expressing vector that is presumed to contain a cryptic promoter that regulates EBNA-1 expression (page 199, column 1, lines 8-16 and Fig. 2). The teachings of Carstens et al. meet the recited elements for the vector in the claims. Therefore, Carstens et al. anticipate the instant claimed invention.

Claims 17-22, 25 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Kobayashi et al. (Antisense Research and Development 5:141-148, 1995).

For a composition claim, it should be noted that the intended use is not given any patentable weight in view of the prior art.

Kobayashi et al. disclosed a transient expression assay for selection of effective antisense RNAs using episomal replication of plasmids in COS-7 cells, an African green monkey kidney-derived cell line expressing SV40 large T antigen. Plasmids expressing antisense RNAs for the retinoblastoma gene (Rb-1) mRNA and harboring SV40 *ori* were constructed and introduced into COS-7 cells to examine their inhibitory effect on the accumulation of endogenous Rb protein (See abstract). More specifically, the DNA fragments were inserted in the antisense orientation into the *apaI/XbaI* sites of a pRc/CMV mammalian expression vector containing CMV enhance-promoter unit, an SV40 ori and a bacterial neomycin resistance gene (page 142, column 1, last paragraph). Therefore, Kobayashi et al. anticipate the instant claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-12, 17-24, 27 and 29-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995. PTO-1449 in paper no. 7).

The claims are directed to an *in vitro* method of expressing a DNA in a pluripotent cell, a vector for transfection of a pluripotent cell *in vitro*, an ES, EC or EG cell transfected with the same and an assay for the effect of the presence in a pluripotent cell of a protein or a polypeptide or other product of DNA expression, with specific limitations recited in the claims.

With respect to the enabled scope of the present invention, Gassmann et al. disclosed mouse ES clone 1.19 cells that contain episomal pMGD20neo DNA and express the polyoma large T antigen. These clonal cells further support the replication, episomal maintenance and expression of hygromycin B under the control of the phosphoglycerate kinase promoter of a second plasmid PGKhphΔLT20. The second plasmid also contains the polyoma *ori*, and it does not express the replication factor polyoma large T antigen due to a 1249-bp deletion in the coding region of the large T gene (page 1295, column 1, see entire first paragraph). For further details on the disclosed pMGD20neo and PGKhphΔLT20 vector plasmids, please refer to Fig. 1. Upon selection for hygromycin B-resistant transfected clones, a high yield of transfected

ES 1.19 cells was obtained and this high yield was attributed to the ability of PGKhphΔLT20 to replicate and be maintained as an episome in clone 1.19 cells (page 1295, col. 1, first full paragraph). The disclosed second plasmid vector does not contain an additional DNA sequence encoding for a gene product that is not a selectable marker and that it is operably linked to a promoter for the expression of the gene product in the transfected cells (A different interpretation of the claims). Gassmann et al. also did not specifically teach an expression system wherein three independent vectors with recited limitations were introduced into a cell. However, Gassmann et al. specifically teach that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest could be established and maintained in transfected cells with preexisted or established presence of polyoma large T, e.g., mouse ES clone 1.19 cells that contain episomal pMGD20neo. The capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells (see page 1296, col. 1, bottom of second full paragraph).

Accordingly, it would have been obvious and within the scope of skill for a person of ordinary skill in the art at the time of invention was made to modify the disclosed method taught by Gassmann et al. by transfecting vectors (second and third) containing a polyoma *ori* for episomal replication and maintenance, a different selection marker for selection purpose, and a gene of interest operably linked to a promoter for expression in ES clone 1.19 cells previously transfected with pMGD20neo plasmid to arrive at the instant invention. One of ordinary skilled in the art would have been motivated to carry out the above modification because Gassmann et al. suggested that the capability to

establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells (see page 1296, col. 1, bottom of second full paragraph). Furthermore, with respect to the limitations recited for the vector of the present invention, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the disclosed PGKhphΔLT20 vector plasmid of Gassmann et al by incorporating a DNA sequence that encodes for a non-marker gene product operably linked to a promoter in the vector plasmid at the expense of the modified polyoma large T antigen encoding DNA sequence. One of ordinary skilled artisan would have been motivated to carry out the above modification because Gassmann et al. specifically teach that plasmid(s) containing a polyoma ori, a selectable marker and any other gene(s) of interest (the modified plasmid vector) could be established and maintained in transfected cells with preexisted or established presence of polyoma large T, e.g., mouse ES clone 1.19 cells that contain episomal pMGD20neo plasmid, and that the capability to establish plasmids as episomes in ES cells should find utility for a variety of studies of gene regulation in these cells.

Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 1 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) in view of Cooper (U.S. Patent No. 5,624,820).

The claims are drawn to an *in vitro* method of expressing a DNA in a pluripotent cell with the recited steps in claim 1; the same wherein the promoter is inducible.

The teachings of Gassmann et al. have been discussed above. However, Gassmann et al. do not specifically teach the use of an inducible promoter in their disclosed episomal plasmid vector system. At the time of the present invention, Cooper teaches an episomal plasmid vector system in human cells, wherein the episomal plasmid can contain an inducible promoter such as a metallothionein promoter (col. 11, lines 34-48).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vector system taught by Gassmann et al. by introducing an inducible promoter into the episomal plasmid as taught by Cooper. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a control in the expression of genes of interest to study their involvement in the differentiation of mouse ES cells *in vitro* as suggested by Gassmann et al. (page 1296, col. 2, first paragraph).

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 1 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) in view of Carstens et al. (Gene 164:195-202, 1995).

The claims are drawn to an *in vitro* method of expressing a DNA in a pluripotent cell with the recited steps in claim 1; the same wherein transcription of the DNA can be activated by a site specific recombinase.

The teachings of Gassmann et al. have been discussed above. However, Gassmann et al. do not specifically teach the use of a site specific recombinase to regulate the transcription of the DNA in their disclosed episomal vector system. At the time of the present invention, Carstens et al. teach the use of *lox*P sites in their episomal vector system for rapid manipulation of the recovered vectors without the use of restriction enzymes (See abstract).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vector system taught by Gassmann et al. by introducing *lox*P sites into the episomal plasmid vector as taught by Carstens et al., such that the transcription of the DNA in the vector can be activated in the presence of *Cre* recombinase. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a better control in the expression of genes of interest to study their involvement in the differentiation of mouse ES cells *in vitro* as suggested by Gassmann et al. (page 1296, col. 2, first paragraph).

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 17 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carstens et al. (Gene 164:195-202, 1995) or Kobayashi et al. (Antisense Research and Development 5:141-148, 1995) in view of Cooper (U.S. Patent No. 5,624,820).

The claims are drawn to a vector for transfection of a pluripotent cell *in vitro* having the limitations recited in claim 17; the same wherein the promoter is inducible.

With respect to the enabled scope of the present invention, both Carstens et al. and Koybayashi teach episomal plasmid vector systems, in which the disclosed vectors have the limitations recited in claim 17. However, none of the reference specifically discloses that the utilized vectors contain an inducible promoter. At the time of the present invention, Cooper teaches an episomal plasmid vector system in human cells, wherein the episomal plasmid can contain an inducible promoter such as a metallothionein promoter (col. 11, lines 34-48).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the episomal plasmid vectors taught by Carstens et al. and Koybayashi et al. by introducing an inducible promoter into the disclosed episomal plasmids as taught by Cooper. One of ordinary skilled in the art would have been motivated to carry out the above modification in order to have a better control in the expression of the genes of interest in the vectors, cDNA inserts or antisense constructs, in the cases of Carstens et al. and Koybayashi et al., respectively.

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 33 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7) as applied to claims 1-12, 17-24, 27 and 29-35 above, and further in view of Carstens et al. (Gene 164:195-202, 1995) or Cooper (U.S. Patent No. 5,624,820).

The claims are drawn to a method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the method of claim 33.

The teachings of Gassmann et al. have been discussed above. However, Gassmann et al. do not specifically teach the use of their disclosed extrachromosomal plasmid vector system for screening a library of cDNAs. At the time of the present invention, both Carstens et al. and Cooper independently teach the use of episomal vector systems for cDNA library cloning and *in vitro* expression of heterologous genes in cells (See abstract in Carstens et al. article and col. 4, lines 9-14 of the Cooper patent).

Accordingly, it would have been obvious for an ordinary skilled artisan to adapt the episomal plasmid vector system taught by Gassmann et al. for screening a library of cDNAs as taught by both Carstens et al. and Cooper. One of ordinary skilled in the art would have been motivated to adapt the vector system of Gassmann et al. for such a purpose because Carstens et al. noted that the episomal expression system allows high level expression of cDNA libraries and the library vectors can be easily rescued from Hirt-lysates (page 201, col. 1 under Conclusions).

Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Claims 37-40 and 44-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tashiro et al. (Science 261:600-603, 1993, PTO-1449 in paper no. 7) in view of Carstens et al. (Gene 164:195-202, 1995), Gassmann et al. (Proc. Natl. Acad. Sci. 92:1292-1296, 1995, PTO-1449 in paper no. 7), Williams et al. (Nature 336:684-687, 1988) and Moreau et al. (Nature 336:690-692, 1988).

The claims are directed to a method for investigating the properties of a DNA sequence comprising expressing in a pluripotent cell a composite DNA including (a) the DNA molecule under investigation, linked to (b) a DNA coding for a cell active protein; the same method with various limitations recited in the dependent claims.

With respect to the enabled scope of the instant claimed invention, Tashiro et al. taught a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs of the 5' termini of the mRNA (page 600, column 2, first full paragraph). Tashiro taught the construction of the pcDL-SRα-Tac(3') vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. The fusion protein expressed on plasma membranes was detected by antibodies to Tac antigen expressed on the surface of transfected COS-7 cells (See Fig. 1). The pcDL-SRα-Tac(3') vector has cloning sites between the SRα promoter and the coding sequence without a signal

sequence of Tac cDNA (page 600, column 3, lines 3-10). Using this approach, two cDNAs that encode putative cytokine molecules, stromal cell-derived factor-1α (SDF-1α) and SDF-1β were cloned. Tashiro et al. did not teach the expression of the composite DNA in a pluripotent cell or the system wherein the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate or the vector expression system with limitations recited in claim 47.

However, Carstens et al. disclosed a system that allows functional cloning of regulatory genes by the expression of libraries of cDNA inserts either in the sense or antisense direction using Epstein-Barr virus-based shuttle vectors (see abstract). The EBV-based shuttle vector system taught by Carstens et al. is applicable to most mammalian cells with the exception of rodent cells (page 196, column 2, lines 1-2 of the second full paragraph). Additionally, Carstens et al. teach that the use of shuttle vectors offers a number of advantages over other strategies, including, (a) easy recovery of the library vectors from selected clones, (b) expression level of the cDNA expression cassette is unaffected by integration, and (c) no cDNA expression cassettes are lost due to disruption of the vector following integration into genomic DNA (page 196, column 1, last paragraph continues to the top of column 2). Gassmann et al. teach a polyoma virus-based shuttle vector system in which extrachromosomal plasmid vectors can be maintained in mouse ES cells as already discussed above. Gassmann et al. further noted that by analogy with bacteria, the establishment of episomal vectors in ES cells, particularly ones containing DNA segments of interest, may prove to be useful for analyzing and modifying gene expression in ES cells during embryonic development

and possibly in the germ line (page 1292, col. 1, last sentence of first full paragraph). Williams et al. taught that in the presence of a secreted leukemia inhibitory factor (LIF), mouse ES cells retain the stem cell phenotype of compact colonies of small cells with a large nuclear to cytoplasmic ratio *in vitro*, whereas ES cells maintained in normal culture medium without LIF differentiate into colonies containing large, flat differentiated cells over a period of 3-6 days (page 684, column 2, last paragraph continues to top of column 1, page 685). Moreau et al. disclosed the complete cDNA sequence for a secreted LIF (Fig. 1).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the signal sequence trap method disclosed by Tashiro et al. for cloning secreted proteins and type I membrane proteins by expressing the composite DNA in the polyoma virus-based shuttle vector system in mouse ES cells, in which the encoded active cell protein is a leukemia inhibitory factor (LIF) as taught by Gassmann et al., Williams et al., and Moreau et al. to arrive at the instantly claimed invention. One of ordinary skill in the art would have been motivated to carry out the above modification because instead of investigating the properties of a DNA sequence in COS-7 cells, the polyoma virus-based shuttle vector system in mouse ES cells taught by Gassmann et al. offers various advantages of the shuttle vector system as noted above by Carstens et al.. These include a) easy recovery of the library vectors from selected clones, (b) expression level of the cDNA expression cassette is unaffected by integration, and (c) no cDNA expression cassettes are lost due to disruption of the vector following integration into genomic DNA (page 196, column 1,

last paragraph continues to the top of column 2). Additionally, the modified method would allow an easy identification or selection of a mouse ES cell containing a DNA sequence coding for a signal polypeptide on the basis of its induced morphological or proliferative change due to the presence or absence of secreted LIF as taught by Williams et al., and Moreau et al..

Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Conclusions

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Karen Hauda, at (703) 305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Patsy Zimmerman, whose telephone number is (703) 308-0009.

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